FBS10- Microcon Procedure Microconcentration of DNA Samples

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1. Scope

1.1. This procedure is used to concentrate and/or purify the DNA extracted from a sample.

2. Background

2.1. Although microconcentration is incorporated into the organic extraction method(s), it can also be utilized independently as a supplemental technique for concentrating samples with low quantification and/or eliminating possible inhibitors following quantitation. The Millipore Microcon DNA Fast Flow uses a low-binding, anisotrophoic, hydrophilic regenerated cellulose membrane to filter out unwanted particles contained within the sample which are below the size of 100,000 Daltons or 300 single stranded DNA nucleotides (150 double stranded DNA nucleotides). The contents of the filter can then be recovered and reconstituted, generating a cleaner and/or more concentrated sample.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

4. Materials Required

4.1. Microcon DNA Fast Flow centrifugal filter devices

4.2. Microcon tubes

4.3. 1.5 mL microcentrifuge tubes

4.4. TE Buffer

5. Standards and Controls

5.1. If a sample is to be microcon'd, the associated reagent blank must also be microcon'd to the same (or more stringent) concentration levels as the associated sample(s). If a sample needs to be microcon'd and the respective reagent blank has already been microcon'd, an additional negative control (a microcon blank) is to be used. The same volume of liquid used for a sample must also be used for the reagent blank during microconcentration. For example, if two tubes are to be concentrated into one sample, there will be two reagent blanks also concentrated. This will have to be initiated during the extraction phase.

6. Procedures

6.1. Vortex and spin down all the samples to be concentrated, including controls.

6.2. **The order and labeling of initial tubes must be witnessed by a second trained individual.** The witness step will be documented appropriately.

6.3. Obtain and assemble the Microcon device (Microcon DNA Fast Flow device right side up in Microcon tube). Label each tube.

6.4. Add 100 µL TE Buffer to upper reservoir to pre-wet the membrane. Add the DNA sample to the upper reservoir, being cautious to avoid touching the filter with the pipette tip. The sample may be added alone or with additional TE Buffer to a maximum volume of 500 µL. Record the total approximate volume of the sample added to the reservoir (for example, if the extraction volume was 50 µL and 2 µL was used for quantification, the resulting volume would be 48 µL).

6.5. Place the samples in a microcentrifuge for 10-25 minutes at 500 x g. Alternatively, the samples can be centrifuged until the fluid is drawn through the filter.

6.6. Label a new set of Microcon tubes.

6.7. When the fluid has been drawn through the filter, add 10-25 µL of TE buffer to all reservoirs, and invert the upper reservoirs into the Microcon tubes. Microcentrifuge for 3 minutes at 500 x g. Be certain that the caps on the tubes are all facing inward in the microcentrifuge to avoid possible snapping of the caps.

6.7.1. Extended centrifugation (2-3 times longer than guidelines) can lead to dryness. Caution will be taken to prevent the filters from spinning to dryness. If this should occur, vortex for 10-30 seconds after addition of the 10-25 µL of TE buffer, then proceed with recovery.

6.8. Remove and discard the upper reservoir columns from each tube. Measure the amount of sample in the new tubes and record this approximate volume as "volume sample recovered." Appropriately label (i.e. barcode) a set of 1.5 mL tubes. **The order and labeling of the final elution tubes must be witnessed by a second trained individual.** The witness step will be documented appropriately. Transfer the extract from the microcon tube to the newly labeled 1.5 mL microcentrifuge tubes.

6.8.1. **NOTE:** If the microcon procedure was performed to clean the DNA extract, proceed to step 6.9. If the microcon was performed to concentrate the DNA, skip step 6.9 and proceed to step 6.10.

6.8.2. **IMPORTANT:** All negative controls (reagent or microcon blank) must be equal to the lowest volume (most concentrated) of any sample for either the cleanup or concentration step.

6.9. For sample cleanup- add additional TE buffer to the recovered sample to equal the initial volume of the sample recorded in step 6.4 or to desired volume.

6.10. For sample concentration- add additional TE buffer, if necessary, to bring the final sample volume to 32 µL or desired volume.

6.11. The new DNA concentration (C2) can be estimated based on the initial DNA concentration (C1), (if known), the initial volume (V1), and the final volume (V2) using the following formula:

$$C2 = (C1 \times V1) / V2$$

6.12. If the initial quant value is unknown, or undetermined by the first quantitation, then submit an aliquot of the microcon'd DNA, along with an aliquot of the negative control, to quantitation.

7. Sampling

7.1. Not applicable

8. Calculations

8.1. The new DNA concentration (C2) can be estimated based on the initial DNA concentration (C1), (if known), the initial volume (V1), and the final volume (V2) using the following formula:

$$C2 = (C1 \times V1) / V2$$

9. Uncertainty of Measurement

9.1. Not Applicable

10. Limitations

10.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.

10.2. Caution will be taken to prevent microcon filters from exceeding their specified limitations. Excessive g-force may result in leakage or damage to the centrifugal device potentially resulting in loss of DNA.

11. Documentation

11.1. FBU Microconcentration Worksheet (Document Control Number: 1583)

11.2 Applicable Sample Tracking and Control Solutions (STACS) documentation

12. References

12.1. Microcon ® Centrifugal Filter Devices – User Guide

12.2. FBS08 - Organic DNA Extraction

12.3. FBS09 – Differential Organic DNA Extraction

12.4. Forensic Biology Unit Quality Assurance Manual